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"Comparative structural and statistical study of the haemin crystal of different classes of Sub Phylum Vertebrate"

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ABSTRACT

This study presents a comprehensive statistical analysis and comparative structure of hemin crystals across different classes of the subphylum Vertebrata. Hemin, an iron-containing porphyrin derivative, plays a pivotal role in various biological processes, including oxygen transport and enzymatic functions. Hemin crystals are brown, rhombic-shaped crystalline chloride of heme that is used to identify blood stains.

A laboratory study was conducted to assess the structural difference in some common sub vertebrates. Blood samples were obtained from the laboratories, butcher shop and dead animals. This present study gives significant data on Comparative structural size and shape hemin crystals of different classes and statistical data analysis. Various shapes are observed like Spindle with slanting edges, Small broad rectangle with rounded edges, long rectangular with sharp edges, Rhomboidal plates or rectangular. Diverse size where observe, the smallest size of hemin crystals out of the studied species is shown by frog. This research contributes to understanding the biochemical diversity within the Vertebrata and provides insights into the evolutionary significance of hemin in vertebrate physiology.

Key words: Hemin crystal, Vertebrates, Blood, Ludwik Karol.

INTRODUCTION

There are millions of living organisms in this world and are some even yet to be discovered. These living organisms that are known to man, very broadly include animals, plants, microorganisms etc. Allof them show great diversity. Yet, there are some characteristics that are similar between groups of organisms and this is what binds them together. All organisms are classified into three major domains; Archaebacteria, Eubacteria, Eukarya

Earlier there were only 2 kingdoms of plants and animals. Whittaker in 1969 suggested that bacteria should not be in plant kingdom and protozoa not in animal kingdom. He gave the five kingdom classification; Monera, Protista, Fungi, Plantae and Animalia. Kingdom Animalia consists of organisms that range from the simplest of the animal forms to the most complex. The animal kingdom is divided into five infra kingdoms/superphyla, namely Porifera, Ctenophora, Placozoa, Cnidaria and Bilateria. The vast majority of bilaterians belong to two large super phyla: the protostomes, which includes organisms such as arthropods, molluscs, flatworms, annelids and nematodes; and the deuterostomes, which include echinoderms, hemichordates and chordates, the latter of which contains the vertebrates [9].

Vertebrates are the species which have a vertebrae or backbone. The vertebrate animals include reptiles, humans, birds, fish, mammals and amphibians. These are also known as Craniata.Vertebrates are classified into 7 classes based on their anatomical and physiological features.

Jawless Fishes (Class Agnatha), Bony Fishes (Class Osteichthyes), Cartilaginous Fishes (Class Chondrichthyes), Amphibians (Class Amphibia), Reptiles (Class Reptilia), Birds (Class Aves) and Mammals (Class Mammalia)

In vertebrates, the circulatory system is a system of organs that includes the heart, blood vessels, and bloodwhich is circulated throughout the body. Despite significant changes across species in body mass, lifestyle, and anatomy, the physicochemical and biological composition of blood are approximately conserved [3]. Red blood cells (RBCs), the main constituent in addition to plasma, are a common feature to blood for almost all vertebrates. Mammalian RBCs lack nuclei and organelles and are typicallybiconcave disc shaped, with minor exceptions such as camel RBCs which are oblate spheroids [4].

HEMIN CRYSTALS

Ludwik Karol Teichmann first crystallized hemin out of blood in1853. Hans Fischer synthesized hemin, for which he was awarded the Nobel Prize in Chemistry in 1930. Hemin crystals are brown, rhombic-shaped crystalline chloride of heame that are used to identify blood stains. Crystals are homogeneous solids, bounded by plane faces and having a geometric shape. They are also known as Teichmann crystals.

Hemin has the summary formulae $C_{34}H_{32}O_4N_4FeCl$. In the haemoglobin molecule four heame groups are present. Heame is thereby iron protoporphyrin which is present as prostetic group in haemoglobin. It is the heame groups that provide for the red colour of the blood and which are the active parts of haemoglobin for transporting oxygen.Heame is made up of four pyrrole rings and a central iron ion in ferrous state. The hemin crystals are prepared by heating ofthe blood with glacial acetic acid. This ruptures the RBCs and the haemoglobin is released. Also, in this process the ferrous

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form of iron is convertedto ferric form. The globin protein gets denatured by heating with acetic acid; while, heame is converted to oxidized heame called haematin. The haematin combines with halogens such as chloride ions to form insoluble haemin which appear as rhombic crystal of chocolate brown colour [6].

SIGNIFICANCE OF HEMIN CRYSTAL:-

- Hemin crystals are used in forensic and medico-legalpractices to distinguish blood stains from other red-colored stains.
- The hemin test can be performed on dried blood stains.
- It helps to give an opinion as to proof whether a stain is blood or something else.
- It is also useful in the difference of bloods of difference species depending upon the shape of the crystal.

OBJECTIVES

The objectives of this work is to study-

- The specific character of haemin crystal of different classes of subphylum vertebrates.
- Shape of haemin crystal of different classes of subphylum vertebrates.
- Size of haemin crystal of different classes of subphylum vertebrates.
- Comparative structural size and shape of hemin crystals of different classes
- Statistical data analysis.

METHODOLOGY

Sample Collection

Blood samples were obtained from the laboratories, butcher shop and dead animals.

Materials Required

Glassslides, Cover slips, Dropper, Spirit lamp, Cotton, glacial aceticacid, Compound Microscope, Light Microscope

Preparation of Hemin Crystals

A small amount of dry blood is taken on a glass slide and crushed to a fine powder with the help of the fused end of a glass rod or with a needle. One crystal of common salt (NaCI) is added to it, which is also crushed to powder [5]. The two are thoroughly mixed and two drops of glacial acetic acid added to it. The mixture is covered with a coverslip and the slide heated over the flame of a spirit lamp.

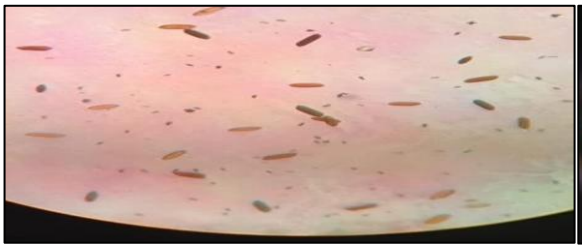
Their action is complete with the beginning of boiling of the mixture and the slide is quickly removed from the flame.The preparation is allowed to cool and examine under a microscope, initially under low magnification and then under high magnification.

STATISTICAL ANALYSIS

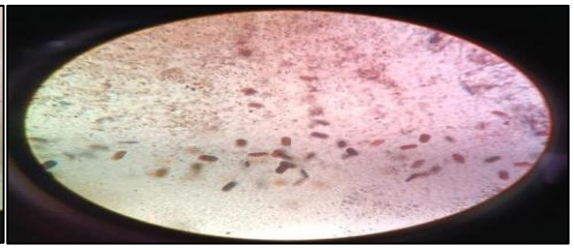
The length and breadth of the hemin crystals of each species belonging to different classes of sub phylum vertebrate was measured. The measurement was done in ocular stage micrometer.

OBSERVATION AND RESULTS

CLASS – PISCES



1. Channa striata **(Osteichthyes)**
(Spindle with slanting edges)



2. Parastromateus niger **(Chondrichthyes)**
(Small broad rectangle with rounded edges)

Tab no 1:-Length and breadth of the hemin crystals of *Channa striata*

Obs. no.	Length (mm)	Breadth (mm)
1	4.5	1
2	5	1.5
3	5	1
4	4	1.3
5	5	1.3

Mean of Length of crystal in *Channa Striata*:-
X=Σx /5 =23.5/5 =4.7 mm
Mean of breadth of crystal in *Channa striata*:-
X= Σx /5 = 6.1 / 5=1.22 mm

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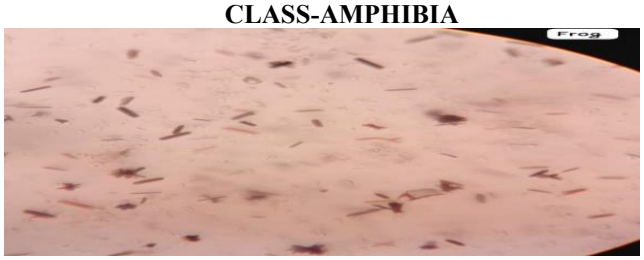
Tab no 2:-Length and breadth of the hemin crystals of *Parastromateus niger.*

Obs no.	Length (mm)	Breadth (mm)
1	6	2
2	6	2.5
3	6	2
4	5	3
5	5.6	2.7

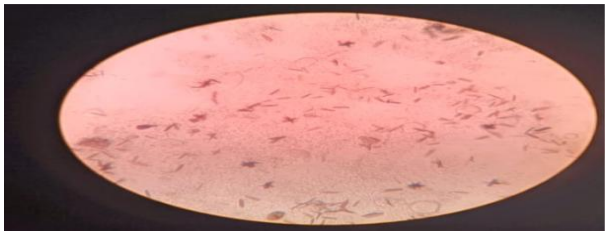
Mean of length= 28.6 /5 = 5.72 mm
Mean of breadth=12.2/5=2.44mm

CLASS-AMPHIBIA



FROG
(Long rectangular with sharp edges)

CLASS: -REPTILIA



LIZARD
(Long sized rectangle with slanting edges)

Tab no 3:- Length and breadth of the hemin crystals of Frog

Obs no.	Length (mm)	Breadth (mm)
1	4	0.8
2	4.5	0.7
3	3.5	0.9
4	3.2	0.5
5	3	0.5

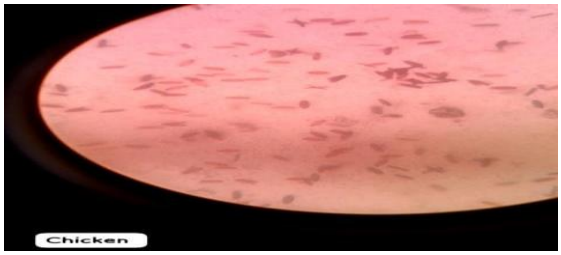
Mean of length = 18.2/5 = 3.64mm
Mean of breadth = 3.4/5 = 0.68mm

Tab no 4:-Length and breadth of the hemin crystal of lizard

Obs no.	Length (mm)	Breadth (mm)
1	10	2
2	11	2
3	12	1.9
4	11	2.5
5	9	3

Mean of length= 53 /5=10.6 mm
Mean of breadth=11.4/5=2.28mm

CLASS AVES:-



(Small broad spindle shaped.)

Tab no 6:-Length and breadth of the hemin crystals of chicken

Obs no.	Length (mm)	Breadth (mm)
1	4	1.1
2	5	1
3	5	1.3
4	4.5	1.9
5	4	1.9

Mean of length = 22.5 /5= 4.5mm
Mean of breadth = 7.2/5 = 1.44mm

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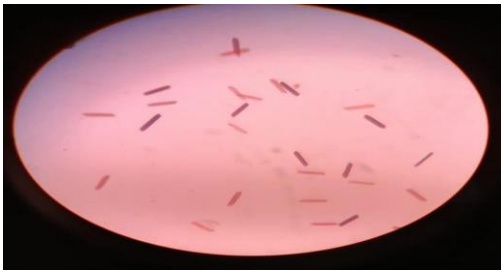
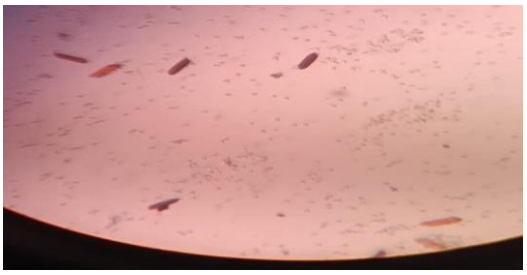
CLASS MAMMALS



Human
(Rectangle with sharp edges, breadth portion Is somewhat inwardly projected)



Goat
(Rhomboidal plates or rectangular)

Tab no7:-Length and breadth of the hemin crystals of human.

Obs no.	Length (mm)	Breadth (mm)
1	8	1.9
2	8.5	1.8
3	9	1.2
4	8	1.1
5	8.5	1.2

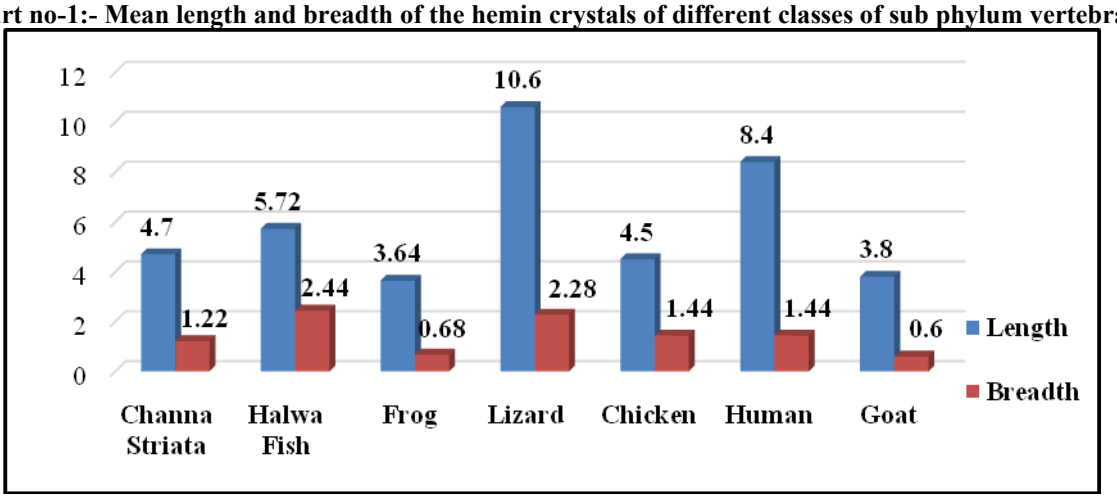
Mean of length= 42 /5 = 8.4 mm
Mean of breadth = 7.2/5=1.44mm

Tab no 8:-Length and breadth of the hemin crystals of goat.

Obs no.	Length (mm)	Breadth (mm)
1	4	0.5
2	4	0.4
3	3.5	0.8
4	4	0.5
5	3.5	0.8

Mean of breadth = 3/5= 0.6mm
Mean of length =19/5=3.8mm

Chart no-1:- Mean length and breadth of the hemin crystals of different classes of sub phylum vertebrate



CONCLUSION

From the comparative study of hemin crystal of the classes of subphylum vertebrates following conclusions can be drawn.

Maximum length out of the studied hemin crystal was 10.6mm found in class Reptilia. Minimum length out of the studied hemin crystal was frog found in class Amphibia. Maximum breadth out of the studied hemin crystal was 2.44mm found in *Parastromateus niger* (Chondrichthyes) of class Pisces. Minimum breadth out of the studied hemin crystal was 0.68 mm frog found in class Amphibia. Thus, it can be concluded that the smallest size of hemin crystals out of the studied species is shown by frog.

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